REMARKS

Claims 1-37 remain under active prosecution in the present application. The preliminary amendments received 02/14/2002 and 04/02/2004 have been entered . Applicant respectfully asserts that all amendments are supported by the original disclosure and do not introduce new matter. Moreover, Applicants further respectfully assert that the amendments merely clarify the scope of the claims.

Specification

In the subject Office Action dated September 8, 2004, the Examiner has pointed out that Table 1 has been amended to include sequence identifiers. However, the various and different sequences all have the same SEQ ID NO: 1 sequence identifier. The specification has now been amended to include the sequence numbering matched with the described sequences. These amendments do not introduce new matter into the disclosure of the invention.

Claim Objections

Claims 11 and 18 are objected to because of the following informalities: Claim 18 is missing a comma and reads "CYP1ACYP2D6" in line 3 rather than "CYP1A, CYP2D6". Claims 11 and 18 have now been amended.

Claim Rejections - 35 USC § 112

The Examiner has rejected claims 1-37 under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The Examiner contends that the claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The claims are drawn to methods of measuring contaminants in water comprising introducing into a transgenic aquatic organism a DNA construct comprising one or more regulatory response element genes operably linked to a reporter gene, exposing the organism to a water sample to be tested with conditions that permit expression of the reporter gene

expression and correlating the expression to known standards to determine the quantity of contaminants in the water sample.

The Examiner contends that the specification is not enabling for the claimed methods because the specification has failed to teach that the transgene constructs encompassed by the claims are responsive to contaminants *in vivo* when stably incorporated into the genome of a fish or other aquatic organism. The Examiner then points out that the specification has taught a number of constructs that are responsive to contaminants *in vitro* and that the specification has taught using a number of constructs comprising regulatory response elements to make transiently transfected zebrafish (see Table 2, page 26). However, the specification only teaches that the EFl-GFPZ-MTLCR was used to make stable transgenic zebrafish (paragraph 0081). The specification does not teach that the transiently transfected F_0 zebrafish made with any construct other than EFl-GFPZ-MTLCR maintained expression into adulthood or was transmitted through the germline, which would indicate stable incorporation into the genome.

The Applicants respectfully point out that although possible, it is not necessary in order to practice the present invention to construct a stably integrated transgene in the host. The methods of the current invention can be used with transient expression of the transgenes.

While the Examiner contends that unintegrated transgenes are often misregulated and expressed ectopically in undesired tissues to varying levels, this is not a concern in the present invention. All that is required is that a transgene be expressed in the host. Upon transfection, animals will be selected for those with proper expression of the transgene and will be *individually* correlated to a known standard in order to use for detection of a contaminant. Any transgenic animal not expressing the transgene may be discarded. Since each individual host is correlated to a known standard, it is not necessary that the levels of expression in each animal be similar in response. What are used in the present methods are individual animals that are standardized and used over the course of their life-times. Obviously, any individual host that has misregulation is discarded.

The variability of transgene expression and resultant impact on the predictability and reproducibility of phenotype in organisms becomes moot in the present invention. While the Examiner contends that the instant specification has merely taught that the transgenes are expressed in transient transfected embryos and has not taught proper regulation of the genes in response to specific contaminants, the inventors have had success in with many transgenes.

Besides the *AhRDtkluc3*, the inventors have had success with GFP in zebrafish fries (3- to 6-day embryos) treated with dioxin, cadmium chloride, and tert-butylhydroquinone. See attached photographs for results of tests and corresponding conditions.

Claim Rejections - 35 USC § 112-2nd paragraph

The Examiner has rejected claims 1-37 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner contends that claims 1 and 2 are unclear because of the phrase "introducing into a transgenic aquatic organism" in line 3. Claims 3-10 and 13-37 depend directly or indirectly from claim 1. Claims 11 and 12 depend from claim 2.

Applicants appreciate the Examiner's comments pointing out this ambiguity. What is meant is "introducing the construct, which makes the organism transgenic." The claims have now been amended to correct any ambiguity.

The Examiner contends that claims 1 and 2 are unclear because of the phrase "at least one regulatory response element gene". Claims 3-10 and 13-37 depend directly or indirectly from claim 1. Claims 11 and 12 depend from claim 2.

Applicants appreciate the Examiner's comments pointing out this ambiguity. This should be simply "response element". For more than 15 years, it has been known that enhancers will drive (turn on) virtually **any gene** that it is placed nearby. The claims have now been amended to correct any ambiguity.

The Examiner contends that, in claim 2, step a, it is unclear if the step is referring to a single DNA construct having multiple regulatory response elements operatively linked to multiple reporter genes in tandem or if the step is referring to multiple separate constructs, each having a single regulatory element linked to a single reporter gene. Claims 11 and 12 depend from claim 2.

Applicants appreciate the Examiner's comments pointing out this ambiguity. What is meant is multiple enhancer motifs of the same type in tandem, driving one reporter gene. Applicants believe that the term "a DNA construct" adequately describes this as a single entity.

The Examiner contends that claim 2 is unclear because of the phrase "the reporter genes" in

that it is not clear if the phrase "the reporter genes" in lines 12-13 is referring to the same gene as line 5, comprising just the coding sequence, or if it is referring to the entire DNA construct of step (a) including the regulatory elements. Claims 11 and 12 depend from claim 2.

Applicants appreciate the Examiner's comments pointing out this ambiguity. There is only one "reporter gene" in a construct. However, with microinjection, multiple enhancer-reporter gene constructs are often injected and they are inserted, often in tandem, randomly into the host DNA. This would make for increased sensitivity and these would be detected, and controlled for, by standardizing each organism before use in the field. The claims have now been amended to correct any ambiguity.

Claims 3 and 4 recite the limitation "the regulatory response element" in line 1. There is insufficient antecedent basis for this limitation in the claim. Claim 1 refers to "the regulatory response element gene". Claims 5-10 and 13-37 depend from claim 4.

The claims have now been amended to correct for antecedent basis.

The Examiner contends that claim 11 is unclear because it refers to "at least one response element from a gene selected from the group consisting of:"; however, the list of genes also contains elements such as AHRE1, AHRE2 and AHRE5, which are not genes. Claim 12 depends from claim 11.

Applicants appreciate the Examiner's comments pointing out this ambiguity. "AHRE1, 2, and 5" should simply refer to the "AHRE (dioxin-response element)." or the "AHREs (dioxin-response elements)." The claims have now been amended to correct any ambiguity.

The Examiner contends that claims 11 and 18 are unclear because they contain abbreviated gene names. The full gene names cannot be determined based on the claim or the disclosure. For example, it is not clear if ACE1 is meant to represent acetylcholinesterase or angiotensin 1 converting enzyme. Both are abbreviated ACE1 in the art. Claim 12 depends from claim 11. Claims 19-37 depend from claim 18.

Although there are often many synonyms for genes, it is well known in the art that the correct gene nomenclature can always be found at: **http://www.gene.ucl.ac.uk/nomenclature/.** For example, *ACE1* and *ACE2* are two homologous genes encoding for angiotensin-converting enzyme. As shown, the Approved Gene Symbol, *ACE*, represents angiotensin I converting enzyme (peptidyl-dipeptidase A) 1 (the Approved Gene Name), having a Location at 17q23, a

Sequence Accession ID of J04144, a Previous Symbol of DCP1, and Aliases of ACE1 and CD143.

The Examiner contends that claims 13-18 recite the limitation "the transgene" in line 1 and there is insufficient antecedent basis for this limitation in the claim. Claims 19-37 depend from claim 18.

Applicants appreciate the Examiner's comments pointing out this ambiguity. The phrase "the transgene" should simply refer to "the DNA construct." The claims have now been amended to correct any ambiguity.

Claim 19 recites the limitation "the reporter element" in line 1. There is insufficient antecedent basis for this limitation in the claim.

Applicants appreciate the Examiner's comments pointing out this ambiguity. The phrase "the reporter element" should simply refer to "the response element." The claims have now been amended to correct any ambiguity.

Claim 34 is wholly unclear. Claim 34 recites the limitation "the native genes" in line 1. There is insufficient antecedent basis for this limitation in the claim. It is not clear what "the native genes" is referring to. Therefore, no clear interpretation of what the claim encompasses can be made.

Applicants appreciate the Examiner's comments pointing out this ambiguity. The phrase "the transgene" should refer to "the sequence of the response element of the DNA construct" which has a degree of homology to the native sequence of the response element. The claim has now been amended to correct any ambiguity.

Claim 34 recite the limitation "the transgenes" in line 1. There is insufficient antecedent basis for this limitation in the claim. It is unclear if the claim is referring to the entire reporter gene construct, the reporter gene, the promoter, or the response elements.

Applicants appreciate the Examiner's comments pointing out this ambiguity. The phrase "the transgene" should simply refer to "the DNA construct." The claim has now been amended to correct any ambiguity.

Claim 35 is unclear because it recited that the reporter gene has 85% homology to the "luciferase system". It is not clear how a gene can be homologous to a system.

Applicants appreciate the Examiner's comments pointing out this ambiguity. The phrase

"luciferase system " should simply refer to "luciferase gene." The claim has now been amended to correct any ambiguity.

The Examiner contends that claim 36 is unclear because it states that the reporter gene has at least 85% homology to the species Aequorea and that it is not clear how a gene can be homologous to a species.

This should be "the reporter gene has a sequence that is at least 85% homologous to an Aequorea sequence." The claim has now been amended to correct any ambiguity.

Claim 36 is unclear because it refers to "Aequorea" as a species. "Aequorea" is a genus. A species names requires a genus and a specific epithet. The claim has now been amended to correct any ambiguity.

Conclusion

In light of the amendments and remarks made herein, it is respectfully submitted that the claims currently pending in the present application are in form for allowance. Accordingly, reconsideration of those claims, as amended herein, is earnestly solicited. Applicants encourage the Examiner to contact their representative, Stephen R. Albainy-Jenei at (513) 651-6839 or salbainyjenei@fbtlaw.com.

The Assistant Commissioner for Patents is hereby authorized to charge any deficiency or credit any overpayment of fees to Frost Brown Todd LLC Deposit Account No. 06-2226.

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